**Written evidence submitted by Durham University (AMR0025)**

Research submitted by Dr Roger R. Draheim

Lecturer in Microbiology within the Division of Pharmacy, School of Medicine, Pharmacy and Health and Fellow of the Wolfson Research Institute for Health and Wellbeing, Durham University

## 1.0 Executive Summary

1.1 The UK Five Year Antimicrobial Resistance Strategy 2013-2018 is comprehensive with respect to the lack of boundaries of antimicrobial resistance (AMR). Successful implementation will require a global perspective as outlined within the strategy.

1.2 The three Strategic Aims and seven Key Areas for Future Action are comprehensive and plausible. No recommendations for alterations have been suggested.

1.3 Projections based upon historical levels of AMR research funding[1] suggest that the academic research component of the strategy is not feasible. We propose significant increases in AMR-specific funding from the UK government.

1.4 A carefully planned and more thorough public programme focused on the imminent threat posed by AMR should be implemented to raise awareness among the general public and health professionals. In addition, this public programme should include a component that brings the challenge of AMR to the forefront of the attention of researchers and students within science, technology, engineering and mathematics (STEM) careers.

## 2.0 Introduction

2.1 Dr Roger R. Draheim is a member of the School of Medicine, Pharmacy and Health and a Fellow of the Wolfson Research Institute for Health and Wellbeing at Durham University. Dr Draheim's research interests lie in reducing the cost required to discover new antibiotics. His work focuses on the application of synthetic bacterial signalling pathways in order to develop a next-generation biological platform for high-throughput detection of compounds with novel antimicrobial activity.

2.2 The School of Medicine, Pharmacy and Health has an active international research programme which emphasises practical issues of translating research into high quality care. The Wolfson Research Institute for Health and Wellbeing exists to foster and disseminate the wide range of research undertaken at Durham University to improve human health and wellbeing.

2.3 This submission provides the Committee with evidence of current developments in the application of synthetic biology to combat increasing antibacterial resistance and highlights the advantages of these approaches in enabling the development of new antibiotics.

## 3.0 Research and investment into new antibiotics to ensure continued protection against infection

3.1 Multidrug resistance (MDR) is a frequent problem in several clinical pathogens. In Europe, antibacterial-resistant infections kill nearly 25,000 patients and represent a total expenditure of approximately £1.5 billion per year.[2] However, given the expensive research, development and clinical testing required to bring an antibacterial to market,

coupled with the fact that they are taken for limited time courses and not for life, makes them a very unattractive prospect for pharmaceutical companies.

3.2 There is a compelling case to develop new technologies drawing on synthetic biological processes in order to reduce the expense associated with discovery of new antibiotics and increase accessibility to novel antibacterials. According to the recently published Synthetic Biology Roadmap for the UK, the field has the potential to "deliver important new applications and improve existing industrial processes – resulting in significant economic growth and job creation."[3] The report highlights the UK's early role in responding to opportunities in synthetic biology as well as its international influence.

3.3 Within this context there is evidence to support the inhibition or overstimulation of two-component systems (TCSs) as an unrealized mechanism of action to harness novel antimicrobials. For example, in several pathogens, host adrenergic signalling molecules, such as epinephrine and norepinephrine, have been shown to upregulate[1] virulence factor expression via the QseC-QseB TCS. LED209, a small molecule compound**,** has been shown to *inhibit* this upregulation in several animal models.[4] Conversely, peptidoglycan recognition proteins (PGRPs), which are part of the host innate immune system and function in antimicrobial immunity, have been shown to *overstimulate* the CpxA-CpxR system of *Escherichia coli* and the CssR-CssS system of *Bacillus subtilis*.[5] These two studies demonstrate that small molecules that *inhibit* or *overstimulate* TCSs are excellent candidates for novel antimicrobials.

3.4 TCSs are composed of modular protein domains and allow bacteria to perceive environmental stimuli and respond accordingly. To control a diverse array of bacterial processes, canonical TCSs must detect a wide variety of stimuli. Within an evolutionary context, this has been accomplished by altering its stimulus-sensing properties and DNA-binding specificity, while leaving the remainder of the TCS scaffold fundamentally unchanged. Two-component systems (TCSs) are abundant in bacteria and notably absent in humans and other animals, however, they remain largely untapped as potential antibacterial targets. Dr Draheim's current research activity harnesses this conservation within a novel "biological screening" platform that physically couples the extracellular domains from targeted TCSs to specific intracellular domains that govern precise intracellular signalling pathways.

3.5 The QseC, CpxA and CssR prot**e**ins described above contain an extracellular domain where interaction occurs to *inhibit* (QseC) or *overstimulate* (CpxA and CssR) signal output to reduce pathogenicity or kill microbial cells, respectively. The proposed "biological platform" is derived from rewiring of bacterial signalling circuits by creating chimeric receptors composed of an extracellular stimulus-perceiving domain from one receptor and the cytoplasmic signalling domain from another (see Section 6.0). The novel part of the research is that Dr Draheim's research group found a way to connect these extracellular domains to a well-characterized intracellular domain of a different protein (EnvZ). When this well-characterized intracellular domain detects an interaction with the extracellular domain**,** fluorescent genes are transcribed which results in the *E. coli* cells in which the "screening" **occurs to fluoresce.** This fluorescence can be detected

[1] A process to make pathogens more virulent.

inexpensively, rapidly and in parallel (**using a** 96-well plate format). For example, this allows compounds like LED209 to bind to a chimeric QseC-EnvZ that will cause *E. coli* cells to become fluorescent when LED209 interacts with the extracellular domain (derived from QseC) of the chimera. It is important to understand the scalability of this technique**:** Dr Draheim's research group is explicitly designing this biological platform (fluorescent *E. coli* cells expressing chimeric proteins) to be compatible with the majority of TCSs in nature. Currently, roughly 200,000 TCSs have been identified within more than 7**,**500 sequenced microbes resulting in an average of more than 25 targetable TCSs per microbe.[6,7]

3.6 There are many potential advantages to such an approach, including:

3.6.1 Efficiency and cost: There are already in existence a wide number of chemical libraries, including small molecule libraries ranging in size from approximately 300 to 1.6 million samples (with the largest of these in Ukraine)[8]. As stated above, an average microbe possesses approximately 25 TCSs**.** Therefore, screening entire smaller libraries or smaller selections of larger libraries against all TCSs in a pathogenic organism become feasible with minimal expense. Using these existing libraries would drastically reduce the typical costs associated with identification of novel antimicrobials while increasing the rate at which potential antimicrobial targets can be subjected to small molecule libraries.

3.6.2 Specificity: One large advantage of this methodology is that only the targeted TCSs of interest will provide a response (i.e. only interactions with the specific extracellular domain of the chimeric protein will result in the cells becoming fluorescent). Standard screening methods result in identification of compounds that inhibit microbial growth but usually do not instantly provide information about the direct target of the antimicrobial.

3.6.3 Safety: One significant advantage to this methodology is that initial screening can be performed in the absence of the pathogenic microbe. This will greatly reduce the initial cost of screening because antimicrobial-resistant organisms could be screened under general laboratory conditions. As an example, *Staphylococcus aureus* subsp. *aureus MRSA252*, which has been sequenced and possesses 18 TCSs,[6,7] create chimeric proteins with an EnvZ intraceullar domain as described above and express them in the "biological platform" that consists of standard laboratory grade *E. coli* cells. This allows these 18 TCSs to be subjected to small molecule libraries without the need to initially grow meticillin-resistant staphylococcus aureusis **(**MRSA). Subsequently, a database of small molecules that demonstrate interaction with the chimeric protein in the biological platform, referred to as "hits", can then be tested within the actual MRSA.

4.0 Strengths and weaknesses of the Government's five year strategy for tackling antimicrobial resistance

4.1 Strengths

We believe that the UK Five Year Antimicrobial Resistance Strategy 2013-2018 is well written, thorough and precisely detailed. In addition, the strategy captures the importance of AMR to human health. Specific examples include:

4.1.1 A clear and succinct statement that few public health issues are of greater importance

4.1.2 Identification that AMR is a global issue that requires action at the local, regional, national and global levels

4.1.3 A correct statement that AMR cannot be eradicated and that its development and spread can only be slowed

4.1.4 Use of an elegant tripartite approach of understanding AMR, conservation and stewardship of existing treatments, and stimulation of new antibiotics, diagnostics and therapies

4.1.5 A direct statement that this is an ambitious approach and that UK government cannot succeed in isolation

4.1.6 Establishment of an interdepartmental High-Level Steering Group (HLSG)

4.1.7 A recommendation that clinicians, veterinarians and other healthcare professionals need to work in closer collaboration with industry

4.1.8 Development of a strategy that will bring about fundamental changes in approach and capability that will extend well beyond the five-year term**.**

4.2 Weaknesses

Although the strategy is well-planned and thoroughly considered, and the global ramifications of AMR are precisely described, we believe that several weaknesses still exist:

4.2.1 The reasons that negatively affect antimicrobial discovery and development, including that fact that it currently takes between 10 and 15 years to bring a novel antibiotic to market and that a relatively low commercial return on investment relative to other therapies, are not described in detail. Although potential remedies to issues are proposed in Sections 4.15 and 4.16 of the Strategy, they appear either inadequate to address the financial issues underpinning these concerns or operate on a time scale too long to have a meaningful impact within the timescale of the strategy (e.g. regulatory reform).

4.2.2 The funding totals provided under Section 2.7 of the Strategy, which, hopefully are not exhaustive, represent a total expenditure of approximately £40m between 2008 and 2015, equates to £5m per year, which is woefully inadequate to tackle the required research to effectively combat AMR. When

employing the FEC (Full Economic Cost) model used by most major research universities in the UK, a postdoctoral researcher with no experience costs roughly £40K/year, which indicates that he funding described in Section 2.7 could fund roughly 125 personnel throughout the entire UK over a given year.

4.2.3 However, this is not a realistic estimate as it does not consider the costs of the required research infrastructure or consumables. From a personal perspective, a recent proposal to the BBSRC to fund the research outlined about for 3 years had a FEC of approximately £500k over three years for a single full-time postdoctoral researcher. At those rates, the projected £5m will fund roughly 35 full-time researchers across the UK.

4.2.4 It should be stated that underfunding of AMR is not a new issue. A comprehensive review of research funding concerning infectious disease was recently conducted.[1] This review found that out of 6,165 funded projects representing £2.6b in total expenditure between 1997 and 2010, that only £106m (or less than four per cent) was directed at antimicrobial research in general, including antiviral and antifungal research (not only resistance to antibiotics). Furthermore, when research involving the three most funded fields (HIV, tuberculosis and malaria) are removed, that number plummets to £62.5m or approximately three per cent of total research funding over the 14 year period, which when inflation is taken into account, is consistent with the roughly £5m/year detailed in Section 2.7 of the strategy. Given my personal calculations as detailed above, this should result in approximately 35 full-time personnel spread throughout the entire UK, which is woefully inadequate to address the scientific issues detailed in the strategy.

## 5.0 Recommendations and action points

We believe that the Government should:

5.1 Continue to follow the outlined strategy as it is well-planned and comprehensive.

5.2 Increase research funding to a level that is consistent execution of the outlined research strategy.

5.3 Implement a carefully planned and more thorough public programme focused on the imminent threat posed by AMR. In addition, this public programme should include a component that brings challenge of AMR to the forefront of the attention of researchers and students within STEM careers.

## 6.0 Relevant Publications (within the last 5 years)

Draheim, R. R.*, Nørholm, M. H. H., Botelho, S. C., Enquist, K. and von Heijne, G.* (2013) Aromatic tuning facilitates stimulus-independent modulation of the EnvZ*Ec*/OmpR osmosensing circuit, *in revision at Molecular Microbiology*

Adase, C. A., Draheim, R. R., Desai, R., Rueda, G., and Manson, M. D. (2013) Residues at the Cytoplasmic End of Transmembrane Helix 2 Determine Signal Output of the Tar*Ec* Chemoreceptor, *Biochemistry 52,* 2729-38

Adase, C. A., Draheim, R. R., and Manson, M. D. (2012) Residue composition of the aromatic anchor of TM2 regulates the signaling state of the aspartate/maltose chemoreceptor Tar of *E. coli*, *Biochemistry 51,* 1925-32.

Tarry, M., Skaar, K., von Heijne, G., Draheim, R. R. and Högbom, M. (2012) Production of human tetraspanin proteins in Escherichia coli. *Protein Expres Purif 82*, 373-9.

Unnerståle, S., Mäler, L.*, and Draheim, R. R.* (2011) Structural characterization of AS1-membrane interactions from a subset of HAMP domains. *BBA Biomembranes 1808*, 2403-12.

Wright, G. A., Crowder, R.L., Draheim, R. R., and Manson M. D. (2011) Mutational Analysis of the TM2-HAMP connection in Tar*Ec*, the E. coli Aspartate Receptor. *J Bacteriol 193,* 82-90.

Wagner, S., Klepsch, M. M., Schlegel, S., Appel, A., Draheim, R., Tarry, M., Högbom. M., van Wijk, K.J., Slotboom, D.J., Persson, J.O., and de Gier, J.W. (2008) Tuning *Escherichia coli* for Membrane Protein Overexpression, *Proc Natl Acad Sci U S A 105,* 14371-6.

Lai, R.Z., Bormans, A.F., Draheim, R. R., Wright, G.A., and Manson, M.D. (2008) The Region Preceding the C-terminal NWETF Pentapeptide Modulates Baseline Activity and Aspartate Inhibition of *Escherichia coli* Tar, *Biochemistry 47,* 13287.

## 7.0 References


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2. Kirby, T. (2012). Europe to boost development of new antimicrobial drugs. *The Lancet* 379 (9833), pp.2229-2230.
3. Technology Strategy Board (2012). *A synthetic biology roadmap for the UK*. Available online: http://www.rcuk.ac.uk/documents/publications/SyntheticBiologyRoadmap.pdf [Accessed 6 November 2013]
4. Rasko, D.A., Moreira, C.G., Li de, R., Reading, N.C., Ritchie, J.M., Waldor, M.K., Williams, N., Taussig, R., Wei, S., Roth, M., Hughes, D.T., Huntley, J.F., Fina, M.W., Falck, J.R., Sperandio, V. (2008). Targeting QseC Signaling and Virulence for Antibiotic Development. *Science* 321 (5892), pp. 1078-80.

5. Kashyap, D.R., Wang, M., Liu, L.H., Boons, G.J., Gupta, D. and Dziarski, R. (2011). Peptidoglycan recognition proteins kill bacteria by activating protein-sensing two-component systems. *Nat Med* 17 (6), pp. 676-83.
6. Ulrich, L.E, Zhulin, I.B. (2010). MIST2: a comprehensive genomics resource on microbial signal transduction. *Nucleic Acids Research* 38. Available online: http://nar.oxfordjournals.org/content/38/suppl_1/D401.full [Accessed 6 November 2013]
7. Publicly available Mist 2.2 database available at http://mistdb.com
8. Hamon, V., Brunel, J.M., Combes, S., Basse, M.J., Roche, P. Morelli, X. (2013). 2P2Ichem: focused chemical libraries dedicated to orthosteric modulation of protein-protein interactions. *Med Chem Comm* 4, pp.797-809.

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